

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for expanding cytotoxic lymphocytes which comprises:

culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2,

wherein the recombinant fibronectin fragment is

a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19,

wherein said culturing is performed for 2-15 days,

wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment,

and wherein said cytotoxic activity of the expanded cytotoxic lymphocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphocyte.

2. (Previously Presented) The method according to claim 1, wherein the expanded cytotoxic lymphocytes highly express an interleukin-2 receptor at a higher level than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment.

3. (Previously Presented) The method according to claim 1, wherein the expanded cytotoxic lymphocytes express more CD8 than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment.

4. (Canceled).

5. (Previously Presented) The method according to claim 1, wherein said at least one recombinant fibronectin fragment is immobilized on a solid phase.

6. (Previously Presented) The method according to claim 5, wherein the solid phase is a cell culture vessel or a cell culture carrier.

7. (Previously Presented) The method according to claim 6, wherein the cell culture vessel is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane or a slide glass.

8. (Withdrawn) The method according to claim 1, wherein expanding a cytotoxic lymphocyte is performed in a cell culture medium comprising said recombinant fibronectin fragment or a mixture thereof.

9. (Cancelled)

10. (Previously Presented) The method according to claim 1, wherein the at least one recombinant fibronectin fragment has cell adhesion activity and/or heparin binding activity.

11. (Cancelled)

12. (Previously Presented) The method according to claim 1, comprising:
expanding a cytotoxic lymphocyte in a cell culture in the presence of said at least one recombinant fibronectin fragment,

wherein at least (a) or (b) is true:

(a) a ratio of the number of cells present at the initiation of the cell culture to a cell culture area is 1 cell/cm^2 to $5 \times 10^5 \text{ cells/cm}^2$; and

- (b) a concentration of cells at the initiation of the cell culture is from 1 cell/ml to 5×10^5 cells/ml.

13. (Cancelled)

14. (Withdrawn) A cytotoxic lymphocyte obtained by the method of claim 1.

15. (Withdrawn) A medicament comprising as an effective ingredient a cytotoxic lymphocyte obtained by the method of claim 1.

16. (Withdrawn) An agent for enhancing an interleukin-2 receptor expression of a cell, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

17. (Withdrawn) The agent according to claim 16, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

18. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

19. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

20. (Withdrawn) An agent for improving a ratio of CD8-positive cell in a lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment

thereof or a mixture thereof.

21. (Withdrawn) The agent according to claim 20, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

22. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

23. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

24. (Withdrawn) An agent for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

25. (Withdrawn) The agent according to claim 24, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

26. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

27. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment is a

polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

28. (Currently Amended) A method for increasing expression of an interleukin-2 receptor in cytotoxic lymphocytes, which comprises:

culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, thereby increasing expression of interleukin-2 receptor in the cells,

wherein the recombinant fibronectin fragment is

a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19,

wherein said culturing is performed for 2-15 days,

wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment,

and wherein said cytotoxic activity of the expanded cytotoxic lymphocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphocyte.

29. (Currently Amended) A method for increasing the number of CD8-positive cells in cytotoxic lymphocytes, which comprises:

culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, thereby increasing the number of CD8-positive cells in the

cultured cells,

wherein the recombinant fibronectin fragment is
a polypeptide comprising an amino acid sequence selected from the group consisting of
SEQ ID NOS: 1 to 19,

wherein said culturing is performed for 2-15 days,
wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than
cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment,
and wherein said cytotoxic activity of the expanded cytotoxic lymphocyte is evaluated
as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining
fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic
lymphocyte.

30. (Canceled).

31. (Previously Presented) The method according to claim 1, further comprising
transducing a foreign gene into the cytotoxic lymphocytes.

32. (Original) The method according to claim 31, wherein the foreign gene is transduced
using retrovirus, adenovirus, adeno-associated virus or simian virus.

33. (Previously Presented) The method according to claim 1, wherein an expansion ratio
of the cytotoxic lymphocytes is high as compared to that of a method for expanding cytotoxic
lymphocytes in the absence of at least one fibronectin fragment.

34. (Previously Presented) The method according to claim 1, wherein expanding
cytotoxic lymphocytes is performed in the presence of both of said at least one recombinant
fibronectin fragment and an anti-CD3 antibody.

35. (Previously Presented) The method according to claim 1, wherein expanding

cytotoxic lymphocytes is performed by incubating peripheral blood mononuclear cells or umbilical cord blood mononuclear cells.

36. (Cancelled).

37. (New) The method according to claim 1, wherein the fluorescent substance is calcein-AM.

38. (New) The method according to claim 28, wherein the fluorescent substance is calcein-AM.

39. (New) The method according to claim 29, wherein the fluorescent substance is calcein-AM.